

# Effect of Temperature on Viability of *Campylobacter jejuni* and *Campylobacter coli* on Raw Chicken or Pork Skin<sup>†</sup>

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## ABSTRACT

To determine growth and survival of *Campylobacter jejuni* and *Campylobacter coli* on chicken and pork, *Campylobacter* spp. ( $10^4$  CFU/cm<sup>2</sup>) were inoculated on pieces of raw, irradiated chicken or pork skin and exposed to temperatures ranging from  $-20$  to  $42^\circ\text{C}$  under either microaerobic or aerobic conditions. Viable counts over 48 h declined 2 to 3 log CFU/cm<sup>2</sup> at  $-20^\circ\text{C}$  and 1 to 2 log CFU/cm<sup>2</sup> at  $25^\circ\text{C}$  regardless of skin type, species of *Campylobacter*, or level of oxygen. At  $4^\circ\text{C}$ , there was no significant change in the number of *Campylobacter* over 48 h. At both 37 and  $42^\circ\text{C}$ , the number of viable *Campylobacter* increased significantly (2 to 3 log CFU/cm<sup>2</sup>,  $P < 0.0001$ ) under microaerobic conditions but decreased 0.5 to 1.5 log CFU/cm<sup>2</sup> in air. Preincubation of skins for 24 h at  $42^\circ\text{C}$  under microaerobic conditions to establish *Campylobacter* on the surface prior to lowering the temperature to  $-20$ , 4, or  $25^\circ\text{C}$  and incubating in air resulted in a decline in viability for the first 4 h (0.5 to 1 log CFU/cm<sup>2</sup>). However, after this initial drop in viability, no additional effect on viability was observed compared with incubation at  $-20$ , 4, or  $25^\circ\text{C}$  in air without microaerobic preincubation at  $42^\circ\text{C}$ . Preincubation of inoculated skins at  $-20$ , 4, or  $25^\circ\text{C}$  in air for 24 h followed by a shift in temperature to  $42^\circ\text{C}$  for 4, 8, 24, or 48 h and a shift to microaerobic conditions resulted in an overall decline in viability on raw pork skin but not on raw chicken skin. In contrast, preincubation of inoculated skins at  $-20$ , 4, or  $25^\circ\text{C}$  for 24 h in air followed by a shift in temperature to  $37^\circ\text{C}$  and microaerobic conditions did not result in a decrease in viable counts for either chicken or pork skins. Overall, viability of *C. coli* and *C. jejuni* on chicken and pork skins was similar. Therefore, a lower incidence of *Campylobacter* spp. in pork than in poultry postslaughter, despite a similar prevalence in live animals, is not due to differences in viability of *C. coli* versus *C. jejuni* on raw chicken or pork skin.

The most common etiology of bacterial gastroenteritis in the world is infection by *Campylobacter* spp. (12). Approximately two million cases of campylobacteriosis are reported each year in the United States alone, and the majority of these cases are sporadic (19). In sporadic cases, it is often difficult to find the original source of infection; however, these cases have been principally attributed to cross-contamination from raw meat or to incomplete cooking of meat (19). The species of *Campylobacter* most frequently associated with human illness are *Campylobacter coli* and *Campylobacter jejuni*. In developed countries such as the United States, more cases of campylobacteriosis are caused by *C. jejuni*, whereas *C. coli* causes more cases of campylobacteriosis in underdeveloped countries (8, 18, 21). The incidence of *C. coli* might be underestimated in the United States because many strains of *C. coli* are more sensitive than *C. jejuni* to antibiotics used in selection media (7, 10, 13, 14).

*Campylobacter* spp. are prevalent in chickens and pigs

(prevalence  $>80\%$ ) (9, 17); however, raw pork has a contamination rate of 3 to 6%, whereas, 60% of retail raw chickens are contaminated (19). This discrepancy in contamination rates between raw pork and raw chicken, despite a similar prevalence in live animals, might be due to differences in the slaughtering process, to differences in viability of *Campylobacter* on different types of meat, or to differences in the survival rates of different *Campylobacter* species. *C. coli* is the predominant species in swine, and in some areas, swine are the source of unusually high proportions of *C. coli* infection in humans (19). *C. jejuni* has also been isolated from pigs, although it is most often associated with chickens.

Although typical food storage conditions are not associated with the growth of *Campylobacter* (5, 15), the length of time at which cells can survive at ambient and refrigerated storage temperature is significant, because the infectious dose can be as low as 500 cells (1, 16). Prior to cooking, meat is exposed to many changes in temperature ranging from freezing temperatures ( $-20^\circ\text{C}$ ) to abuse temperatures ( $25^\circ\text{C}$ ), and viability of *Campylobacter* at a specific temperature following a temperature shift can be different than viability at that temperature in the absence of a shift.

In this study, viability of *C. jejuni* and *C. coli* on pieces of raw irradiated chicken or pork skin was assessed at  $-20$ , 4, 25, 37, and  $42^\circ\text{C}$ . Four sets of experiments were con-

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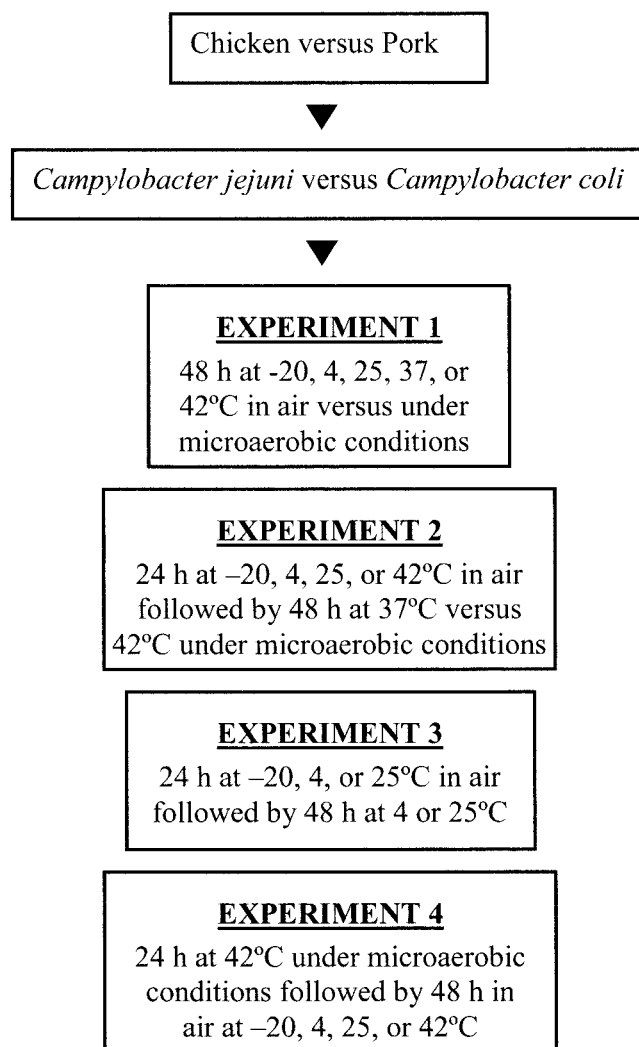


FIGURE 1. Flow diagram of experiments conducted to examine viability of *C. jejuni* and *C. coli* on chicken and pork skins.

ducted (Fig. 1). These studies were conducted to determine whether (i) viability of *C. coli* differed from that of *C. jejuni*; (ii) viability differed on pieces of raw chicken skin versus raw pork skin; (iii) survival on exposure to suboptimal temperatures differed for *Campylobacter* already growing on raw skins versus *Campylobacter* not yet established on skins; (iv) changes in storage temperature affected viability; and (v) survival of *Campylobacter* following cold stress was affected by either the storage temperature or the recovery temperature.

## MATERIALS AND METHODS

**Growth of bacterial strains.** The strains used in this study were *C. jejuni* ATCC 33560 (American Type Culture Collection, Manassas, Va.; bovine), ATCC 35918 (ovine), and ATCC 35922 (human) and *C. coli* strains 5164 (National Animal Disease Center, Ames, Iowa; poultry) and ATCC 33559 (pig). The strains were routinely cultured on modified charcoal cefoperazone desoxycholate agar (mCCDA, Oxoid Ltd., Basingstoke, Hampshire, England) at 42°C in sealed jars under a microaerobic atmosphere generated by CampyPak Plus gas generators (BBL, Cockeysville, Md.). Cultures were passed onto fresh mCCDA every 2 days.

**Preparation of chicken and pork skins.** Raw chicken and pork skins were provided by local commercial poultry and pork processing plants. The skins were irradiated at the Eastern Regional Research Center (Wyndmoor, Pa.) in a Lockheed Georgia irradiator, model 3060 ( $^{137}\text{Cs}$  source) at a dosage of 25 kGy and a temperature of  $-20^{\circ}\text{C}$ . Skins were stored at  $-20^{\circ}\text{C}$  prior to use. Before each experiment, the skins were cut aseptically using sterile scalpels into 2-cm<sup>2</sup> pieces weighing approximately 2 g each. For each experiment, sterilization of the skins was confirmed by incubating 1-cm<sup>2</sup> pieces of skin in duplicate at 37°C under microaerobic and aerobic conditions for the duration of the experiment. The skins were placed in 10 ml of 0.1% peptone (Becton Dickinson, Sparks, Md.) and macerated in a stomacher (Lab-Blender, Tekmar Co., Cincinnati, Ohio); then, 0.2 ml of the mixture was plated onto mCCDA and tryptic soy agar (Becton Dickinson). The mCCDA was incubated at 37°C for 48 h under microaerobic conditions, and the tryptic soy agar was incubated at 37°C for 48 h in air. Sterility of the raw pieces of skin was determined by absence of growth on either the mCCDA or the tryptic soy agar plates.

**Growth of *C. jejuni* or *C. coli* on raw pieces of skin.** For each time point and temperature, the three strains of *C. jejuni* or the two strains of *C. coli* were harvested from mCCDA and serially diluted in 0.1% peptone to a final concentration of  $10^5$  to  $10^6$  CFU/ml, containing equal levels of each strain as determined by OD<sub>600</sub>. Each cocktail of strains was then inoculated in 0.2-ml aliquots onto the surface of duplicate pieces of skin in sterile petri dishes (100 by 15 mm). Petri dishes containing inoculated skins were incubated at the temperatures and lengths of time indicated (Fig. 1). For incubation in air, petri dishes were wrapped in Parafilm to prevent drying. For incubation under microaerobic conditions, petri dishes were incubated with CampyPaks in sealed jars. To determine the number of viable *Campylobacter*, skins were macerated in 10 ml of 0.1% peptone for 2 min in a stomacher, and the homogenate was then serially diluted and spread in duplicate onto mCCDA. The mCCDA plates were incubated under microaerobic conditions for 48 h at 42°C, and the tryptic soy agar plates were incubated for 48 h at 30°C. The inoculum also was serially diluted and plated onto mCCDA to determine the number (CFU/ml). At least two replications of each experiment were done using duplicate pieces of skin for each time point.

**Viability of *C. jejuni* versus *C. coli* on pieces of raw chicken skin versus raw pork skin.** *C. jejuni* and *C. coli* were inoculated onto pieces of raw chicken or pork skin. The pieces of skin were incubated at  $-20$ , 4, 25, 37, or 42°C in air for 0, 4, 8, 24, or 48 h or under microaerobic conditions for 0, 4, 8, 24, or 48 h. Viable counts were then determined as described above.

**Comparing survival of *Campylobacter* already growing on skins to *Campylobacter* not yet established on skins.** One set of raw chicken and pork skin pieces was inoculated and then incubated at 42°C under microaerobic conditions for 24 h, followed by incubation in air at  $-20$ , 4, or 25°C or under microaerobic conditions at 42°C for 4, 8, 24, or 48 h. Another set of raw skin pieces was inoculated then immediately incubated in air at  $-20$ , 4, or 25°C or under microaerobic conditions at 42°C for 4, 8, 24, or 48 h. Viable counts were then determined as described above.

**Determining whether changes in storage temperature affect viability of *Campylobacter*.** Raw chicken and pork skin pieces inoculated with *C. jejuni* or *C. coli* were incubated in air at  $-20$ , 4, or 25°C for 24 h. For each temperature ( $-20$ , 4, or 25°C), the pieces of skin were divided into two sets. One set was incubated in air at 4°C for an additional 4, 8, 24, or 48 h, and the

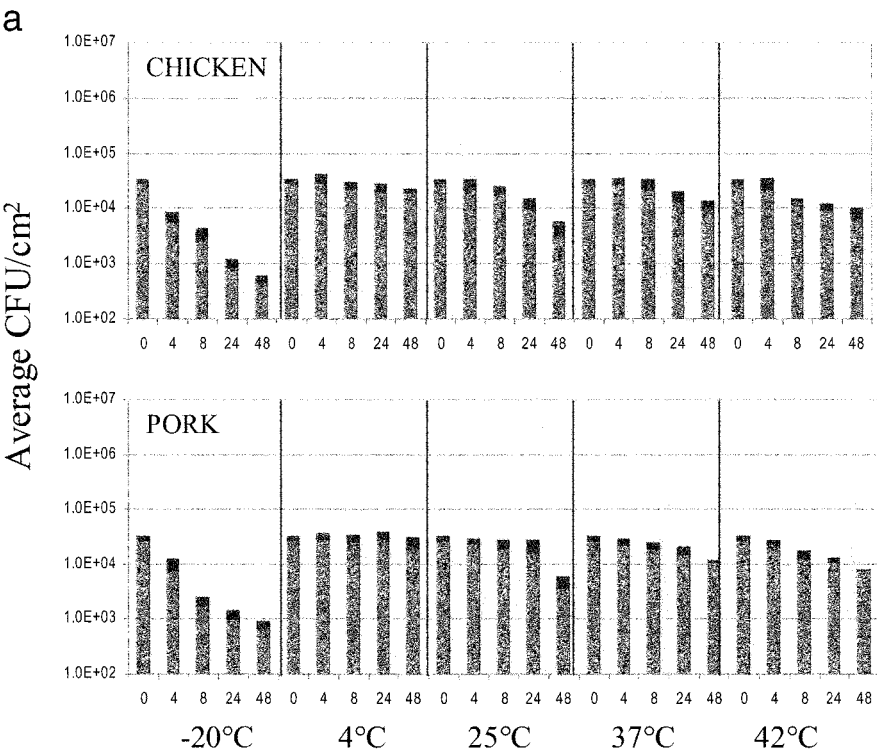
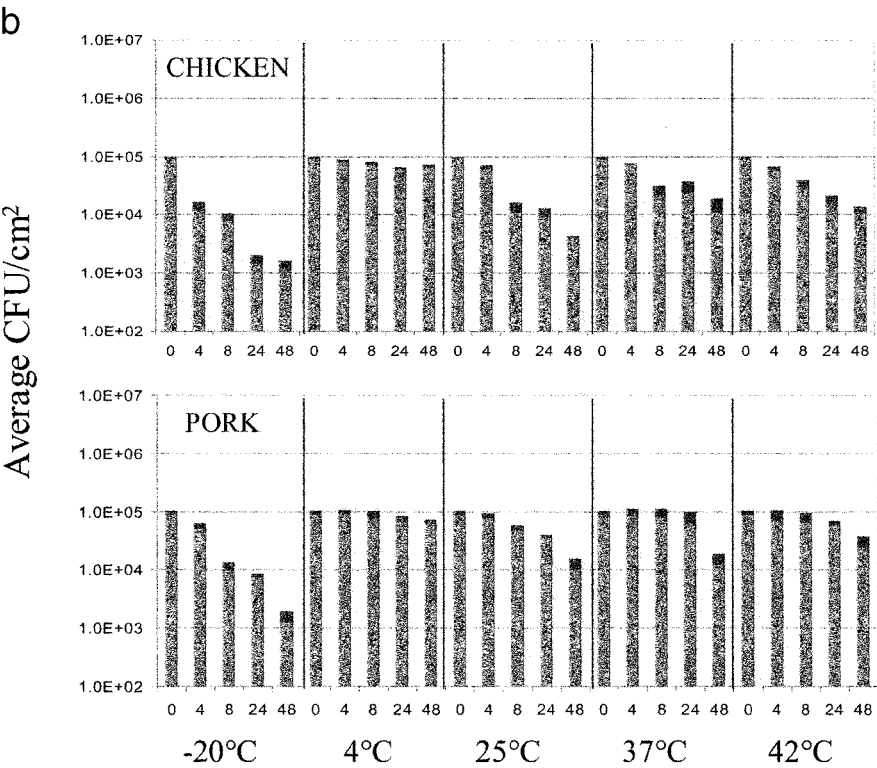


FIGURE 2. Viability of *C. jejuni* (a) and *C. coli* (b) on irradiated chicken and pork skins incubated at  $-20$ ,  $4$ ,  $25$ ,  $37$ , or  $42^{\circ}\text{C}$  in air. Results are shown as the average  $\pm$  standard deviation.



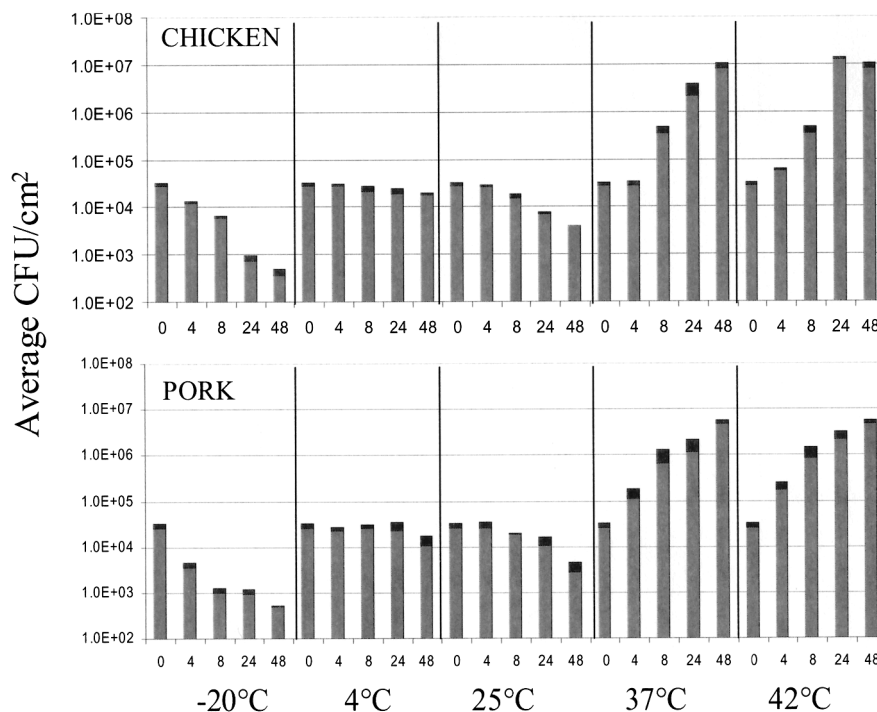
other set of skins was incubated in air at  $25^{\circ}\text{C}$  for an additional 4, 8, 24, or 48 h, for a total incubation time of 72 h. Viable counts were then determined as described above.

**Evaluating the effect of cold stress on the recovery of *Campylobacter*.** Chicken and pork skins inoculated with either *C. jejuni* or *C. coli* were incubated in air at  $-20$ ,  $4$ ,  $25$ ,  $37$ , or  $42^{\circ}\text{C}$  for 48 h. Following the 48-h incubation, the skins incubated at  $-20$ ,  $4$ , and  $25^{\circ}\text{C}$  were divided into two sets. One set of duplicate chicken and pork skins was incubated at  $37^{\circ}\text{C}$  under microaerobic

conditions for an additional 4, 8, 24, or 48 h. The second set of duplicate skins was incubated at  $42^{\circ}\text{C}$  under microaerobic conditions for an additional 4, 8, 24, or 48 h. The skins incubated in air at  $37$  and  $42^{\circ}\text{C}$  were switched to microaerobic conditions and incubated for an additional 4, 8, 24, or 48 h. Viable counts were then determined as described above.

**Electron microscopic examination of skins.** Inoculated ( $10^5$  CFU/cm<sup>2</sup> of *C. jejuni* or *C. coli*) or uninoculated, nonirradiated, raw pork or chicken skin pieces were incubated for 24 h at  $-20$ ,

FIGURE 3. Results of incubation at  $-20$ ,  $4$ ,  $25$ ,  $37$ , or  $42^{\circ}\text{C}$  of irradiated chicken or pork skins inoculated with *C. jejuni* under microaerobic conditions. Results are shown as the average  $\pm$  standard deviation.



$4$ , or  $25^{\circ}\text{C}$  in air or at  $42^{\circ}\text{C}$  under microaerobic or aerobic conditions. Skins were transferred to a solution containing 2.5% glutaraldehyde and 0.1 M imidazole (pH 7.2) and fixed in an  $\text{OsO}_4$  solution. Fixed samples were washed with 0.1 M imidazole, pH 7.2, and dehydrated in a series of increasing concentrations of ethanol from 30 to 100%. Dehydrated samples were dried using liquid  $\text{CO}_2$ , placed in a desiccator overnight, mounted on aluminum stubs, and coated with gold by direct current sputtering. Skins were viewed and photographed with a Joel 840A scanning electron microscope.

**Statistical analyses.** Experiments were performed in duplicate with at least two replicates for each condition. Data were analyzed to test the effect of incubation time, temperature, species, and type of skin and their interactions on bacterial counts. Counts were transformed to the common log, and the four factors (time, temperature, species, and skin type) were analyzed by analysis of variance. When results were significant, a pairwise comparison was made of the data, adjusting the experimental error using the Bonferroni method of adjustment.

## RESULTS

**Viability of *C. jejuni* versus *C. coli* on pieces of raw chicken skin versus raw pork skin.** When comparing survival of *C. jejuni* (Fig. 2a) to *C. coli* (Fig. 2b) on raw chicken or pork skin incubated in air, no significant difference was observed between the two species of bacteria or between the two types of skin for a specific temperature and time point. At all temperatures except  $4^{\circ}\text{C}$ , viable counts of *Campylobacter* decreased over 48 h. The greatest reduction in viability was observed for raw pork or chicken skin incubated at  $-20$  and  $25^{\circ}\text{C}$ . At  $-20^{\circ}\text{C}$ , CFU declined significantly  $1.70 \pm 0.15$  log CFU/cm $^2$  ( $P < 0.001$ ) over 48 h, and at  $25^{\circ}\text{C}$ , CFU declined  $0.91 \pm 0.08$  log CFU/cm $^2$  over 48 h. A slight reduction in viability also was observed at 37 and  $42^{\circ}\text{C}$ . A decrease of  $0.44 \pm 0.08$  log CFU/cm $^2$  was observed at  $37^{\circ}\text{C}$ , and  $0.60 \pm 0.10$  log CFU/cm $^2$  was observed at  $42^{\circ}\text{C}$ .

Although the decline in viability at 37 and  $42^{\circ}\text{C}$  was not statistically significant, it was reproducible. At  $4^{\circ}\text{C}$ , numbers of viable *Campylobacter* did not significantly change over 48 h ( $P = 1.000$ ). Regardless of species or skin type, *C. jejuni* or *C. coli* inoculated onto skins exposed to air survived best at  $4^{\circ}\text{C}$ .

Under microaerobic conditions at  $-20$ ,  $4$ , and  $25^{\circ}\text{C}$  (Fig. 3), the reduction in CFU was similar to that observed under aerobic conditions, but at 37 and  $42^{\circ}\text{C}$  under microaerobic conditions, viability of *C. jejuni* significantly increased ( $P < 0.0001$ ) to a maximum concentration of  $6.78 \pm 0.16$  log CFU/cm $^2$  and  $6.88 \pm 0.30$  log CFU/cm $^2$ , respectively, over 48 h. Viability of *C. coli* was examined under identical conditions in parallel experiments, and no significant difference was observed between *C. jejuni* and *C. coli* at the same time points and temperatures (data not shown). If skins were inoculated with a 10-fold greater number of *Campylobacter* and incubated at  $42^{\circ}\text{C}$  under microaerobic conditions, viable counts peaked at  $7.40 \pm 0.23$  log CFU/cm $^2$  by 24 h then significantly declined ( $P < 0.0001$ ) to  $3.44 \pm 0.77$  log CFU/cm $^2$  over the next 24 h (data not shown). For viability of *C. jejuni* and *C. coli* on raw skin pieces, no significant difference was observed between raw chicken and pork ( $P > 0.11$ ) or between *C. jejuni* and *C. coli* ( $P > 0.13$ ).

**Comparing survival of *Campylobacter* already growing on skins to *Campylobacter* not yet established on skins.** *Campylobacter* are endemic to livestock, are known to live in the intestines of pigs and chickens, and can be established on the skin prior to slaughter. *Campylobacter* found on skins postslaughter have one of two origins. They were either attached to and growing on the skins preslaughter and were not removed during slaughter, or they contaminated the carcasses during the slaughter process.

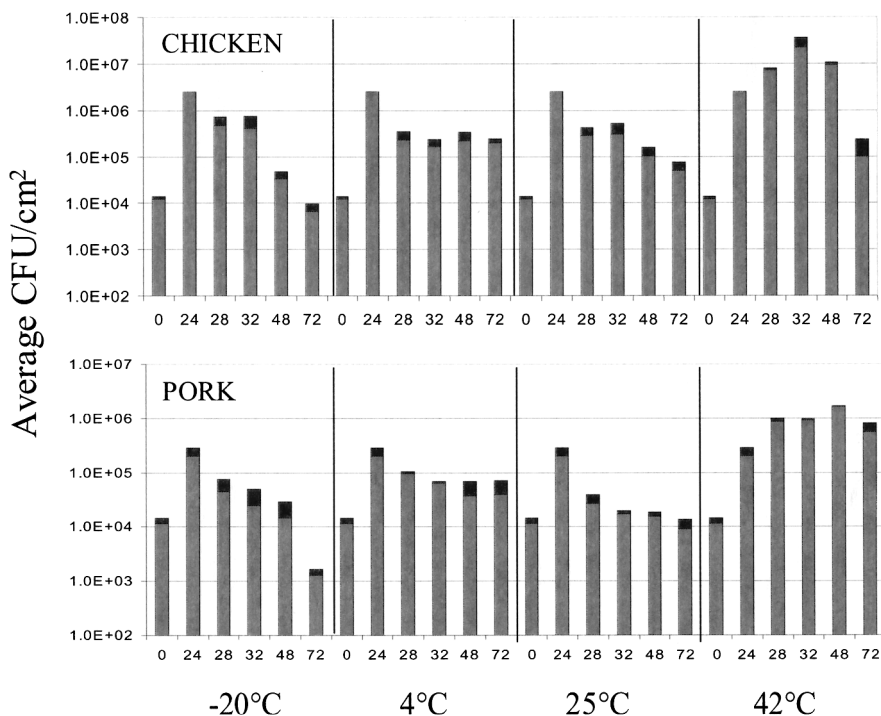


FIGURE 4. Incubation of *C. jejuni* on irradiated chicken or pork skins at 42°C under microaerobic conditions for 24 h, followed by a switch to -20, 4, or 25°C in air or 42°C under microaerobic conditions for 48 h. Results are shown as the average  $\pm$  standard deviation.

cess. Survival of *Campylobacter* on exposure to suboptimal temperatures might be different for *Campylobacter* already growing on skins than for *Campylobacter* not yet established on skins. The experiments described below compare survival of *Campylobacter* at -20, 4, or 25°C for *Campylobacter* previously growing on skins at 42°C versus freshly inoculated onto raw skin pieces.

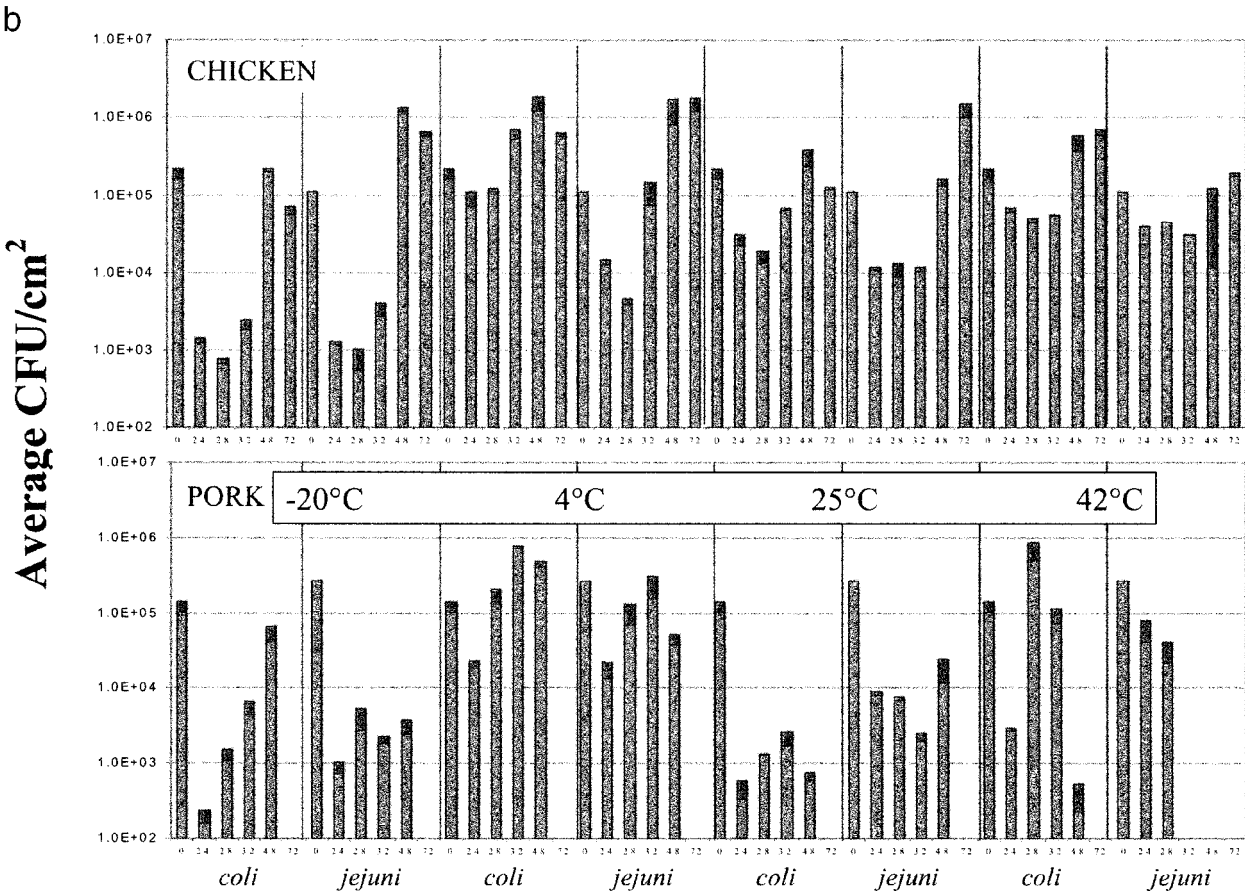
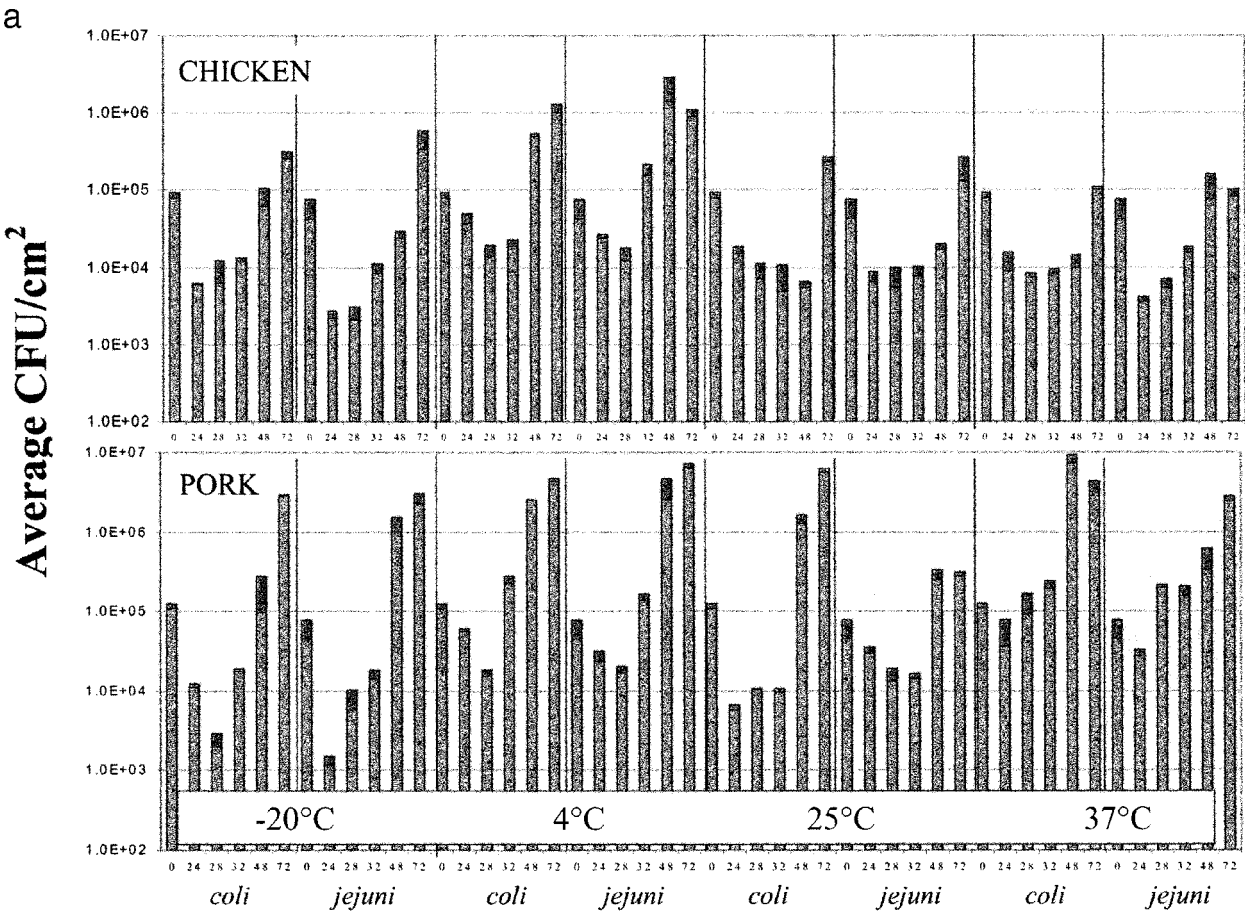
The number of *C. jejuni* on the raw skin pieces significantly increased 1.7 log CFU/cm<sup>2</sup> ( $P < 0.0001$ ) for the first 24 h at 42°C. Following the temperature change from 42°C to -20, 4, or 25°C, viability of *C. jejuni* declined 0.5 to 1 log CFU/cm<sup>2</sup> over 4 h, although over the 48 h at the lower temperatures, viability was not significantly different for *C. jejuni* incubated on the skins at 42°C prior to exposure to suboptimal temperatures versus *C. jejuni* inoculated onto skins and then immediately exposed to the same suboptimal temperatures (data not shown). The overall change in viability of *Campylobacter* was not significantly different ( $P > 0.26$ ) for *C. jejuni* (Fig. 4) versus *C. coli* (data not shown).

**Determining whether changes in storage temperature affect viability of *Campylobacter*.** Before cooking, meat is exposed to many changes in temperature, ranging from freezing temperatures (-20°C) to abuse temperatures (25°C). Viability of *Campylobacter* following temperature shifts from -20, 4, or 25°C to 4 or 25°C was studied to determine whether shifting the temperature resulted in a change in viability at the new temperature, which was different than viability at that temperature in the absence of a shift. The rate of decline in viable counts at the new temperature ( $0.25 \pm 0.04$  log CFU/cm<sup>2</sup>/48 h at 4°C and  $1.22 \pm 0.10$  log CFU/cm<sup>2</sup>/48 h at 25°C) was not significantly different from the rate of decline in viable counts on skins incubated at a fixed temperature of 4 or 25°C ( $0.21 \pm 0.06$  log CFU/cm<sup>2</sup>/48 h and  $1.05 \pm 0.11$  log CFU/cm<sup>2</sup>/48 h,

respectively; data not shown). Thus, the rate of decline in *Campylobacter* viability on chicken and pork skins is not significantly different at 4 or 25°C regardless of the temperature (-20, 4, 25°C) prior to exposure to 4 or 25°C.

**Evaluating the effect of cold stress on the recovery of *Campylobacter*.** The viability of *Campylobacter* on pieces of raw chicken or pork skin under microaerobic conditions at 37 and 42°C following incubation at -20, 4, 25, 37, or 42°C was measured to analyze the recovery of *Campylobacter* after exposure to storage temperatures. After switching to a microaerobic atmosphere and changing the temperature to 37 or 42°C to mimic ideal growth conditions, growth initially lagged at all temperatures, but by 32 h, viable counts started to increase on both chicken and pork skins incubated at 37°C (Fig. 5a) and 42°C (Fig. 5b). On chicken skins incubated at 37 and 42°C and on pork skins incubated at 37°C, viable counts continued to increase to  $6.17 \pm 0.58$  log CFU/cm<sup>2</sup>. In contrast, on pork skins incubated at 42°C, viable counts rapidly dropped to below detectable levels, even though the number of bacteria on the skins had not reached 6.2 log CFU/cm<sup>2</sup>. This resulted in a significant decrease ( $P < 0.001$ ) in viable counts on pork skins compared with chicken skins at 48 and 72 h for skins initially incubated at -20, 25, and 42°C. Therefore, on chicken skins, viability of *C. jejuni* or *C. coli* did not differ at 37°C versus 42°C; however on pork skins, viable counts of *Campylobacter* that had been exposed to -20, 4, and 25°C increased and remained elevated for the entire 48 h when skins were incubated at 37°C but not at 42°C.

**Examination of chicken and pork skins by electron microscopy.** The structure of the pork and chicken skins was different. Chicken skins had many folds and deep crevices with structures projecting inward from the skin surface (Fig. 6a), whereas the pork skins were bumpy with struc-



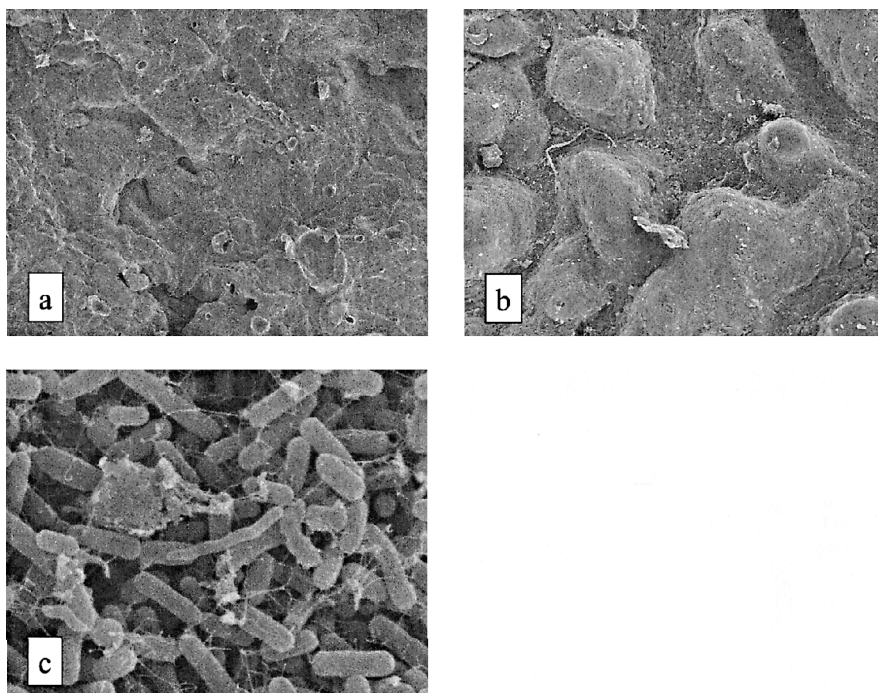


FIGURE 6. Electron micrographs of non-irradiated skins inoculated with *C. jejuni* and incubated for 24 h. (a) Chicken skin incubated at 4°C,  $\times 500$  magnification, image width 230  $\mu\text{m}$ ; (b) pork skin incubated at 4°C,  $\times 500$  magnification, image width 230  $\mu\text{m}$ ; and (c) chicken skin incubated at 42°C,  $\times 5,000$  magnification, image width 23  $\mu\text{m}$ .

tures projecting outward from the skin surface (Fig. 6b). At 42°C under microaerobic conditions, *C. jejuni* (Fig. 6c) or *C. coli* (data not shown) grew in colonies composed of a mixed population of bacterial species. In contrast, at  $-20$ , 4, and 25°C, *Campylobacter* were in small groups and were predominantly isolated from other bacteria on both chicken skins and pork skins (data not shown). The close proximity of *Campylobacter* with other bacteria at 42°C was attributed to the overall increase in the number of *Campylobacter* on skins at 42°C versus  $-20$ , 4, and 25°C.

### DISCUSSION

Before cooking, meat is most typically exposed to temperatures ranging from  $-20$  to 25°C. In the present studies, viability of *C. jejuni* and *C. coli* on chicken and pork skins was examined at three temperatures in this range. Temperatures of  $-20$ , 4, and 25°C were representative of freezer storage, refrigerator storage, and room temperature, respectively. Of these three temperatures, viability of *C. jejuni* and *C. coli* was maintained at the highest level at 4°C, with no difference in viability between *C. jejuni* and *C. coli* or on chicken skin versus pork skin. From a meat processing and production standpoint, maintenance of viability at 4°C is problematic because most meat is processed and stored at 4°C. At 25°C, viable counts declined 1 to 2 log CFU/cm<sup>2</sup> over 48 h, and the decline in viable counts was most dramatic at  $-20$ °C (2 to 3 log CFU/cm<sup>2</sup>). Again, no difference was observed in viability of *C. jejuni* or *C. coli* on chicken skins versus pork skins.

*Campylobacter* spp. are found in the intestinal tracts

and on the carcass surfaces of swine and chickens, and the bacteria can be transferred from the gastrointestinal tract to skin or other surfaces during slaughter. Both pork and broiler slaughter/processing operations involve stunning/killing, exposure to scalding tanks, evisceration, washing, and chilling. During processing, cross-contamination from contact with equipment or other carcasses or during the evisceration and washing/chilling steps can occur. Pork processing includes scalding-dehairing and singeing steps that result in a decrease in levels of bacteria on the carcasses (4). Also, pork carcasses are generally hung to dry and chill for a longer period of time compared with broiler carcasses; thus, broilers are kept more moist, potentially allowing bacterial levels to reach higher numbers than on pork. Thus, differences in pork and broiler processing might account for the differences in prevalence of *Campylobacter* on pork products compared with poultry; however, differences in prevalence could also be due, in part, to differences in viability of *Campylobacter* on poultry compared with pork.

The change in viability at  $-20$ , 4, or 25°C for *Campylobacter* incubated on skins at 42°C prior to incubation at  $-20$ , 4, or 25°C was not significantly different from the change in viability for *Campylobacter* inoculated on skins and then immediately incubated at the respective temperatures. Therefore, there apparently is little difference in viability if *Campylobacter* are already established on live animals prior to slaughter and then the carcasses are exposed to cold temperatures or if the carcasses are contaminated in one of the processing steps postslaughter and then maintained at refrigeration temperatures.

FIGURE 5. Incubation of *C. jejuni* or *C. coli* on irradiated chicken or pork skins at  $-20$ , 4, 25, or 37°C (a) or  $-20$ , 4, 25, or 42°C (b) for 24 h in air, followed by a switch to microaerobic conditions and by an increase in temperature to 37°C (a) or 42°C (b) for 48 h. Results are shown as the average  $\pm$  standard deviation.



Longer survival of *C. jejuni* at 4°C than at -20 or 25°C has been found for other types of meat and meat products (8). Blankenship and Craven (2) detected less than a 2-log decrease in numbers of *C. jejuni* inoculated at 10<sup>8</sup> CFU/cm<sup>2</sup> into sterile ground chicken meat incubated 18 days at 4°C. However, at 23°C, viable counts declined 2 to 6 log over 18 days. Blaser et al. (3) recovered *Campylobacter* from human feces after 3 weeks at 4°C, but no viable organisms could be recovered after 1 week at 25°C. In untreated water, *Campylobacter* survived better at 4°C than at higher temperatures (11). Therefore, survival of *Campylobacter* at 4°C is not unique to chicken and pork skins. In contrast to our studies using chicken and pork skins, *Campylobacter* inoculated onto beef loins survived better at -18°C (<1 log decrease/cm<sup>2</sup> by 10 d) than at 25°C (>3 log decrease per cm<sup>2</sup> to levels below detection by 10 days) (6). This difference in survival for *Campylobacter* on beef loins might be due to lower levels of moisture on the surface of the beef. *Campylobacter* in ground chicken meat, water, and chicken and pork skins are in very moist environments. Because *Campylobacter* spp. are sensitive to freezing, a freeze-thaw cycle has been proposed as a potential method for reducing *Campylobacter* numbers on meat (20).

*Campylobacter* are microaerophilic, and in the environment and on animal skins, they might occupy niches of the skin where the prevalence of oxygen is lower, such as in association with other oxygen-respiring bacteria. Oxidative stress had no effect on viability at the lower temperatures (-20, 4, and 25°C). However, at the optimum growth temperatures of 37 and 42°C, viability of both *C. coli* and *C. jejuni* on chicken and pork skins declined in air, whereas there was growth under microaerobic conditions. *Campylobacter* spp. are viable for a shorter time at 25°C than at 4°C (2, 3, 8, 11). This phenomenon has been attributed to an increase in oxidative stress on the organism at 25°C than at 4°C. At 25°C, *Campylobacter* should have a higher metabolic rate than at 4°C and, therefore, should be more sensitive to oxidative stress. Experiments herein refute this hypothesis because the decline in viability at 25°C was no different in a microaerobic environment than in air. Possible causes of this reduction in viability/culturability include buildup of fermentation products, regulated cell death, or entry into a viable but non-culturable state.

*C. jejuni* and *C. coli* survived equally well on chicken and pork skins. Therefore, a lower incidence of *Campylobacter* spp. in pork (3 to 6%) than in poultry (60%) post-slaughter (2), despite a similar prevalence in live animals (>80%), is not due to differences in viability of *C. coli* versus *C. jejuni* on chicken and pork, even though *C. coli* is more prevalent on pork. The surfaces of chicken and pork skins differed, with structures on chicken skins projecting inward and structures on pork skins projecting outward. Therefore, it is feasible that *Campylobacter* spp. are more easily removed from pork skin during processing but become trapped in the layers of chicken skin. Future experiments will examine the effect of moisture levels on survival

at various temperatures and the effect of the presence of other species of bacteria on *Campylobacter* survival.

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